PROTEIN PURIFICATION PROCESS AND PRODUCT

RELATED APPLICATIONS

This application is a continuation-in-part of copending U.S. Patent Application Ser. No. 106,644, filed Dec. 26, 1979, now abandoned, which is a continuation-in-part of U.S. Patent Application Ser. No. 77,710, now abandoned, filed Sept. 21, 1979, which is a continuation-in-part of U.S. Pat. Application Ser. No. 62,374, now abandoned, filed July 31, 1979, which in turn is a continuation-in-part of U.S. Patent Application Ser. No. 963,257, filed Nov. 24, 1978, now abandoned.

BACKGROUND OF THE INVENTION

Purification of proteins has long been a problem in peptide chemistry. Techniques which have been employed include precipitation, gel filtration, ion exchange chromatography, gel electrophoresis, affinity chromatography, and many others too numerous to mention.

Schemes to isolate naturally occurring, high molecular weight proteins which are present in biological samples in extremely low concentrations have involved multi-step procedures utilizing assays of the aforementioned techniques. In a great number of such cases tremendous quantities of crude starting material must be accumulated and processed at high cost and usually with expenditure of great effort due to large losses of product in the later steps of the purification procedure.

A good case in point is the history of the numerous attempts to isolate and characterize interferon. Since its first discovery by Isaacs and Lindenmann in 1957, interferon whether of the leucocyte or fibroblast form has resisted the attempts of researchers at institutions throughout the world spanning over two decades to be isolated as a homogenous peptide in amounts sufficient to allow characterization and identification of its specific biological and chemical properties.

In U.S. Pat. No. 3,699,222, which is directed to Isaacs and Lindenmann's original research with interferon, the purification of the active material is limited to ammonium sulfate precipitation followed by dialysis. Such procedures are relatively non-specific and thus the product obtained thereby is still in an extremely crude state.

A multi-step procedure for purifying interferon is disclosed in U.S. Pat. No. 3,414,651 utilizing selective adsorption on an amorphous, alumino-silicate, elution with iodine or thiocyanate solution, further precipitation of unwanted protein with aqueous HCl and then aqueous NaOH, precipitation of interferon from the basic solution with water-miscible solvents such as methanol, ethanol or acetone and finally chromatography of the redissolved interferon on an anion exchange resin such as DEAE cellulose to produce an interferon whose specific activity is indicated to have been enhanced 6,000 fold by the entire process. Specific interferons exemplified were chick and monkey interferon.

U.S. Pat. No. 3,800,035 describes a method for inducing interferon production in human leukocytes in the absence of serum. The leukocytes are primed with interferon, the serum removed by centrifugation, the white cells suspended in nutrient medium and induced with a suitable inducing agent. A similar disclosure for inducing interferon in cell cultures is provided in U.S. Pat. No. 3,951,740 with the added feature of providing enough L-glutamine during the priming phase to keep the cells in an active metabolic state.

A further purification variation is taught in U.S. Pat. No. 3,975,344 where a crude human fibroblast interferon solution derived from the incubation medium of the cell culture was purified by zonal density gradient ultracentrifugation. This technique was indicated to give higher yields and purification than obtained with the conventional procedure of column chromatography on Sephadex G-100.

Recent scientific papers directed to the purification and attempted characterization of interferons can be summarized as follows:

Knight, E. (1976) "Interferon: Purification and Initial Characterization from Human Diploid Cells," *Proc. Natl. Acad. Sci. U.S.A.* 73,520–523.

Torma, E. T., and Paucker, K. (1976) "Purification and Characterization of Human Leukocyte Interferon Components," *J. Biol. Chem.* 251, 4810–4816.

Bridgen, P. J., Anfinsen, C. B., Corley, L., Bose, S., Zoon, K. C., Ruegg, U. Th., and Buckler, C. E. (1977) "Human Lymphoblastoid Interferon, Large Scale Production and Partial Purification," *J. Biol. Chem.* 252, 6585–6587.

DeMaeyer, J., Tovey, M. G., Gresser, I., and DeMaeyer, E. (1978)
"Purification of Mouse Interferon by Sequential Affinity Chromatography on poly(U) and Antibody-agorose columns," *Nature* 271, 622–625.

Kawakita, M., Cabrer, B., Taira, H., Rebello, M., Slattery, E., Weideli, H., and Lengyel, P. (1978) "Purification of Interferon from Mouse Ehrlich Ascites tumor cells," *J. Biol. Chem.* 253, 598–602.

Berthold, W., Tan, C., and Tan, Y. H. (1978) "Purification and in vitro labeling of interferon from a human fibroblastoid cell line," i J. Biol. Chem. 253, 5206–5212.

Jankowski, W. J., Davey, M. W., O'Malley, J. A., Sulkowski, E., and Carter, W. A. (1975) "Molecular Structure of Human Fibroblast and Luekocyte Interferons: Probe by Lectin- and Hydrophobic Chromatography," *J. Virology* 16, 1124–1130.

Davey, M. W., Sulkowski, E., and Carter, W. A. (1976) "Hydrophobic Interaction of Human, Mouse, and Rabbit Interferons with Immobilized Hydrocarbons," *J. Biol. Chem.* 251, 7620–7625.

Chadha, K. C., Sclair, M., Sulkowski, E., and Carter, W. A. (1978) "Molecular Size Heterogeneity of Human Leukocyte Interferon," *Biochemistry* 17, 196–200.

While several of the above papers contain claims to have purified mouse or human interferons to homogeneity none of the classical proofs of homogeneity of protein materials were given nor were any properties of the allegedly pure compounds described.

The use of high performance liquid chromatography for purification of proteins is generally known in the art. These references specifically describe ion exchange and size exclusion type columns in protein purification. See for example Regnier and Noel, J. Chromatog. Sci. 14, 316 (1976) and Chang et al., Anal. Biochem. 48, 1839 (1976).

The use of LiChrosorb RP-18 (octadecyl bound silica microparticle column) in reverse phase partition chromatography was successfully employed to purify peptides such as β-endorphin. Rubinstein et al. *Proc. Natl. Acad. Sci., U.S.A.* 74, 4969 (1977).